

We Claim:

1. A recombinant human monoclonal antibody that exhibits immunological binding affinity for a hepatitis C virus (HCV) E2 antigen, wherein said monoclonal antibody comprises an amino acid sequence homologous to the binding portion of a human antibody Fab molecule obtained from a combinatorial antibody library.
2. The monoclonal antibody of claim 1, further characterized in that said monoclonal antibody is cross-reactive to HCV E2 antigens from two or more HCV genotypes.
3. The monoclonal antibody of claim 2, further characterized in that said monoclonal antibody is cross-reactive to E2 antigens from HCV genotypes 1a and 2b.
4. The monoclonal antibody of claim 1, wherein said monoclonal antibody has an IgG isotype.
5. The monoclonal antibody of claim 4, wherein the Fab molecule comprises an amino acid sequence homologous to the binding portion of a human $\gamma 1$ heavy chain variable region (V_H).
6. The monoclonal antibody of claim 5, wherein the Fab molecule comprises an amino acid sequence homologous to the binding portion of a human κ light chain variable region (V_L).
7. The monoclonal antibody of claim 6, wherein the Fab molecule comprises the heavy chain

variable region (V_H) amino acid sequence depicted in Figure 1A (SEQ ID NO: 1).

8. The monoclonal antibody of claim 6, wherein the Fab molecule comprises the heavy chain variable region (V_H) amino acid sequence depicted in Figure 1B (SEQ ID NO: 2).

9. The monoclonal antibody of claim 6, wherein the Fab molecule comprises the heavy chain variable region (V_H) amino acid sequence depicted in Figure 1C (SEQ ID NO: 3).

10. The monoclonal antibody of claim 6, wherein the Fab molecule comprises the heavy chain variable region (V_H) amino acid sequence depicted in Figure 1D (SEQ ID NO: 4).

11. The monoclonal antibody of claim 6, wherein the Fab molecule comprises the heavy chain variable region (V_H) amino acid sequence depicted in Figure 1E (SEQ ID NO: 5).

12. The monoclonal antibody of claim 6, wherein the Fab molecule comprises the heavy chain variable region (V_H) amino acid sequence depicted in Figure 1F (SEQ ID NO: 6).

13. The monoclonal antibody of claim 6, wherein the Fab molecule comprises the heavy chain variable region (V_H) amino acid sequence depicted in Figure 1G (SEQ ID NO: 7).

14. The monoclonal antibody of claim 6, wherein the Fab molecule comprises the light chain

variable region (V_L) amino acid sequence depicted in
Figure 2A (SEQ ID NO: 3).

15. The monoclonal antibody of claim 6,
5 wherein the Fab molecule comprises the light chain
variable region (V_L) amino acid sequence depicted in
Figure 2B (SEQ ID NO: 4).

16. The monoclonal antibody of claim 6,
10 wherein the Fab molecule comprises the light chain
variable region (V_L) amino acid sequence depicted in
Figure 2C (SEQ ID NO: 5).

17. The monoclonal antibody of claim 6,
15 wherein the Fab molecule comprises the light chain
variable region (V_L) amino acid sequence depicted in
Figure 2D (SEQ ID NO: 6).

18. The monoclonal antibody of claim 6,
20 wherein the Fab molecule comprises the light chain
variable region (V_L) amino acid sequence depicted in
Figure 2E (SEQ ID NO: 7).

19. The monoclonal antibody of claim 6,
25 wherein the Fab molecule comprises the light chain
variable region (V_L) amino acid sequence depicted in
Figure 2F (SEQ ID NO: 8).

20. The monoclonal antibody of claim 6,
30 wherein the Fab molecule comprises the light chain
variable region (V_L) amino acid sequence depicted in
Figure 2G (SEQ ID NO: 9).

21. The monoclonal antibody of claim 1, further characterized in that said monoclonal antibody has a binding affinity of at least about 1.0×10^7 .

5 22. The monoclonal antibody of claim 21, further characterized in that said monoclonal antibody also binds to the hepatitis C virus (HCV) E1/E2 complex as determined by an ELISA assay.

10 23. The monoclonal antibody of claim 1, wherein said monoclonal antibody comprises a $F(ab')_2$ fragment capable of binding to a hepatitis C virus (HCV) E2 antigen.

15 24. The monoclonal antibody of claim 1, wherein said monoclonal antibody consists essentially of a Fab molecule capable of binding to a hepatitis C virus (HCV) E2 antigen.

20 25. The monoclonal antibody of claim 24, further characterized in that said monoclonal antibody has a binding affinity of at least about 1.0×10^7 .

25 26. The monoclonal antibody of claim 25, further characterized in that said monoclonal antibody also binds to a hepatitis C virus (HCV) E1/E2 complex as determined by an ELISA assay.

30 27. A single chain Fv (sFv) molecule that exhibits immunological binding affinity for a hepatitis C virus (HCV) E2 antigen, wherein said sFv molecule comprises a binding portion formed from amino acid sequences homologous to a human antibody Fab molecule obtained from a combinatorial antibody library.

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28. The sFv molecule of claim 27, wherein said sFv molecule comprises first and second polypeptide domains connected by a linker moiety, wherein the first polypeptide domain includes an amino acid sequence
5 homologous to the binding portion of a $\gamma 1$ heavy chain variable region (V_H) of a human Fab molecule, and the second polypeptide domain includes an amino acid sequence homologous to the binding portion of a κ light chain variable region (V_L) of a human Fab molecule.

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29. The sFv molecule of claim 28, wherein the linker moiety is not derived from an antibody molecule.

30. The sFv molecule of claim 29, wherein the
15 linker moiety comprises a polypeptide linker comprising a third amino acid sequence joining the C-terminus of one polypeptide domain with the N-terminus of the other polypeptide domain.

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31. An isolated nucleic acid molecule comprising:

Sw 31
a first nucleotide sequence encoding a first polypeptide that is homologous to the binding portion of a $\gamma 1$ heavy chain variable region (V_H) of a human Fab
25 molecule that exhibits immunological binding affinity for a hepatitis C virus (HCV) E2 antigen; and

a second nucleotide sequence encoding a second polypeptide that is homologous to the binding portion of a κ light chain variable region (V_L) of a human Fab
30 molecule that exhibits immunological binding affinity for a hepatitis C virus (HCV) E2 antigen.

32. The nucleic acid molecule of claim 31, further comprising:

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5 a third nucleotide sequence encoding a first leader sequence peptide, wherein said third nucleotide sequence is operably linked to the 5' terminus of the first nucleotide sequence and is capable of causing secretion of the first polypeptide when the first polypeptide and the first leader sequence peptide are expressed; and

10 a fourth nucleotide sequence encoding a second leader sequence peptide, wherein said fourth nucleotide sequence is operably linked to the 5' terminus of the second nucleotide sequence and is capable of causing secretion of the second polypeptide when the second polypeptide and the second leader sequence peptide are expressed.

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33. The nucleic acid molecule of claim 32, wherein the third and fourth nucleotide sequences are selected from the group of leader sequences consisting of *omp A* and *pelB*.

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a *gpc*
34. The nucleic acid molecule of claim 31, wherein the first nucleotide sequence is homologous to the sequence depicted in Figure 4A (SEQ ID NO: ²²₁).

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a
35. The nucleic acid molecule of claim 31, wherein the first nucleotide sequence is homologous to the sequence depicted in Figure 4B (SEQ ID NO: ²³₁).

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a
36. The nucleic acid molecule of claim 31, wherein the first nucleotide sequence is homologous to the sequence depicted in Figure 4C (SEQ ID NO: ²⁴₁).

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a
37. The nucleic acid molecule of claim 31, wherein the first nucleotide sequence is homologous to the sequence depicted in Figure 4D (SEQ ID NO: ²⁵₁).

38. The nucleic acid molecule of claim 31,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4E (SEQ ID NO: ¹⁹₁).

5 39. The nucleic acid molecule of claim 31,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4F (SEQ ID NO: ²⁰₁).

10 40. The nucleic acid molecule of claim 31,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4G (SEQ ID NO: ²¹₁).

a
15 41. The nucleic acid molecule of claim 31,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3A (SEQ ID NO: ¹⁵₁).

a
20 42. The nucleic acid molecule of claim 31,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3B (SEQ ID NO: ¹⁶₁).

a
43. The nucleic acid molecule of claim 31,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3C (SEQ ID NO: ¹⁷₁).

25 44. The nucleic acid molecule of claim 31,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3D (SEQ ID NO: ¹⁸₁).

a
30 45. The nucleic acid molecule of claim 31,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3E (SEQ ID NO: ¹⁹₁).

a
35 46. The nucleic acid molecule of claim 31,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3F (SEQ ID NO: ²⁰₁).

47. The nucleic acid molecule of claim 31,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3G (SEQ ID NO: ²¹₁).

5 48. An isolated nucleic acid molecule,
comprising a first nucleotide sequence encoding a first
polypeptide that is homologous to the binding portion of
a $\gamma 1$ heavy chain variable region (V_H) of a human Fab
molecule obtained from a combinatorial library, wherein
10 said Fab molecule exhibits immunological binding affinity
for a hepatitis C virus (HCV) E2 antigen.

49. The nucleic acid molecule of claim 48,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4A (SEQ ID NO: ²²₁).

50. The nucleic acid molecule of claim 48,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4B (SEQ ID NO: ²³₁).

51. The nucleic acid molecule of claim 48,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4C (SEQ ID NO: ²⁴₁).

52. The nucleic acid molecule of claim 48,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4D (SEQ ID NO: ²⁵₁).

53. The nucleic acid molecule of claim 48,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4E (SEQ ID NO: ²⁶₁).

54. The nucleic acid molecule of claim 48,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4F (SEQ ID NO: ²⁷₁).

a 55. The nucleic acid molecule of claim 48,
wherein the first nucleotide sequence is homologous to
the sequence depicted in Figure 4G (SEQ ID NO: ¹⁷₁).

5 56. An isolated nucleic acid molecule,
comprising a first nucleotide sequence encoding a first
polypeptide that is homologous to the binding portion of
a κ light chain variable region (V_L) of a human Fab
molecule obtained from a combinatorial library, wherein
10 said Fab molecule exhibits immunological binding affinity
for a hepatitis C virus (HCV) E2 antigen.

a 15 57. The nucleic acid molecule of claim 56,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3A (SEQ ID NO: ¹⁵₁).

a 20 58. The nucleic acid molecule of claim 56,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3B (SEQ ID NO: ¹⁶₁).

a 25 59. The nucleic acid molecule of claim 56,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3C (SEQ ID NO: ¹⁷₁).

a 30 60. The nucleic acid molecule of claim 56,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3D (SEQ ID NO: ¹⁸₁).

a 35 61. The nucleic acid molecule of claim 56,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3E (SEQ ID NO: ¹⁹₁).

a 62. The nucleic acid molecule of claim 56,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3F (SEQ ID NO: ²⁰₁).

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63. The nucleic acid molecule of claim 56,
wherein the second nucleotide sequence is homologous to
the sequence depicted in Figure 3G (SEQ ID NO: 21).

5 64. An expression vector, comprising the
nucleic acid molecule of claim 31 operably linked to
control sequences that direct the transcription of the
first and second nucleotide sequences whereby said first
and second nucleotide sequences can be transcribed and
10 translated in a host cell.

65. The expression vector of claim 64, wherein
the control sequences are capable of directing the
transcription of the first and second nucleotide
15 sequences in a prokaryotic host cell.

66. The expression vector of claim 64, wherein
the control sequences are capable of directing the
transcription of the first and second nucleotide
20 sequences in a eukaryotic host cell.

67. An expression vector, comprising the
nucleic acid molecule of claim 48 operably linked to
control sequences that direct the transcription of the
25 first nucleotide sequence whereby said first nucleotide
sequence can be transcribed and translated in a host
cell.

68. The expression vector of claim 67, wherein
30 the control sequences are capable of directing the
transcription of the first nucleotide sequence in a
prokaryotic host cell.

69. The expression vector of claim 67, wherein
35 the control sequences are capable of directing the

transcription of the first nucleotide sequence in a eukaryotic host cell.

5 70. An expression vector, comprising the
nucleic acid molecule of claim 56 operably linked to
control sequences that direct the transcription of the
first nucleotide sequence whereby said first nucleotide
sequence can be transcribed and translated in a host
cell.

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71. The expression vector of claim 70, wherein
the control sequences are capable of directing the
transcription of the first nucleotide sequence in a
prokaryotic host cell.

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72. The expression vector of claim 70, wherein
the control sequences are capable of directing the
transcription of the first nucleotide sequence in a
eukaryotic host cell.

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73. A prokaryotic host cell transformed with
the expression vector of claim 65.

25 74. A prokaryotic host cell transformed with
the expression vector of claim 68.

75. A prokaryotic host cell transformed with
the expression vector of claim 71.

30 76. A eukaryotic host cell transformed with
the expression vector of claim 66.

77. A eukaryotic host cell transformed with
the expression vector of claim 68.

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78. A eukaryotic host cell transformed with the expression vector of claim 72.

79. A method of producing a recombinant human Fab molecule, comprising:

(a) providing a population of transformed host cells according to claim 76; and

(b) expressing said recombinant Fab molecule from the expression vector.

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80. A method of producing a recombinant polypeptide having an amino acid sequence homologous to the binding portion of a $\gamma 1$ heavy chain variable region (V_H) of a human Fab molecule, comprising:

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(a) providing a population of transformed host cells according to claim 77; and

(b) expressing said recombinant polypeptide from the expression vector.

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81. A method of producing a recombinant polypeptide having an amino acid sequence homologous to the binding portion of a κ light chain variable region (V_L) of a human Fab molecule, comprising:

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(a) providing a population of transformed host cells according to claim 78; and

(b) expressing said recombinant polypeptide from the expression vector.

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82. A vaccine composition comprising the monoclonal antibody of claim 1 and a pharmaceutically acceptable vehicle.

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83. A vaccine composition comprising the monoclonal antibody of claim 6 and a pharmaceutically acceptable vehicle.

84. A vaccine composition comprising the monoclonal antibody of claim 22 and a pharmaceutically acceptable vehicle.

5 85. A vaccine composition comprising the monoclonal antibody of claim 24 and a pharmaceutically acceptable vehicle.

10 86. A vaccine composition comprising the sFv molecule of claim 27 and a pharmaceutically acceptable vehicle.

15 87. A method for providing an antibody titer to HCV in a mammalian subject, comprising introducing a therapeutically effective amount of the vaccine composition of claim 82 to said subject.

20 88. A method for providing an antibody titer to HCV in a mammalian subject, comprising introducing a therapeutically effective amount of the vaccine composition of claim 83 to said subject.

25 89. A method for providing an antibody titer to HCV in a mammalian subject, comprising introducing a therapeutically effective amount of the vaccine composition of claim 84 to said subject.

30 90. A method for providing an antibody titer to HCV virus in a mammalian subject, comprising introducing a therapeutically effective amount of the vaccine composition of claim 85 to said subject.

35 91. A method for providing an antibody titer to HCV in a mammalian subject, comprising introducing a

therapeutically effective amount of the vaccine composition of claim 86 to said subject.

92. A method of providing passive immunity
5 against HCV infection in a mammalian subject, comprising introducing a therapeutically effective amount of the vaccine composition of claim 82 to said subject.

93. A method of providing passive immunity
10 against HCV infection in a mammalian subject, comprising introducing a therapeutically effective amount of the vaccine composition of claim 85 to said subject.

94. A method of providing passive immunity
15 against HCV infection in a mammalian subject, comprising introducing a therapeutically effective amount of the vaccine composition of claim 86 to said subject.

95. A method of treating a mammalian subject
20 having an HCV infection, comprising introducing a therapeutically effective amount of the vaccine composition of claim 82 to said subject.

96. The method of claim 95, further comprising
25 the step of administering a therapeutically effective amount of α -interferon to the mammalian subject along with said vaccine composition.

97. The method of claim 95, further comprising
30 the step of administering a therapeutically effective amount of Ribavirin to the mammalian subject along with said vaccine composition.

98. The method of claim 96, further comprising
35 the step of administering a therapeutically effective

amount of Ribavirin to the mammalian subject along with said α -interferon and said vaccine composition.

5 99. A method of treating a mammalian subject having an HCV infection, comprising introducing a therapeutically effective amount of the vaccine composition of claim 86 to said subject.

10 100. The method of claim 99, further comprising the step of administering a therapeutically effective amount of α -interferon to the mammalian subject along with said vaccine composition.

15 101. The method of claim 99, further comprising the step of administering a therapeutically effective amount of Ribavirin to the mammalian subject along with said vaccine composition.

20 102. The method of claim 100, further comprising the step of administering a therapeutically effective amount of Ribavirin to the mammalian subject along with said α -interferon and said vaccine composition.

25 103. A binding complex, comprising the monoclonal antibody of claim 1 and a detectable moiety attached thereto.

30 104. A binding complex, comprising the monoclonal antibody of claim 6 and a detectable moiety attached thereto.

35 105. A binding complex, comprising the monoclonal antibody of claim 22 and a detectable moiety attached thereto.

106. A binding complex, comprising the monoclonal antibody of claim 24 and a detectable moiety attached thereto.

5 107. A binding complex, comprising the sFv molecule of claim 27 and a detectable moiety attached thereto.

10 108. The binding complex of claim 103, wherein the detectable moiety is selected from the group consisting of radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors and dyes.

15 109. The binding complex of claim 106, wherein the detectable moiety is selected from the group consisting of radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors and dyes.

20 110. The binding complex of claim 107, wherein the detectable moiety is selected from the group consisting of radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors and dyes.

111. A method for detecting the presence of HCV particles in a sample suspected of containing HCV, comprising:

30 (a) incubating the sample with the binding complex of claim 108, wherein the incubating is conducted under conditions which allow for formation of an antibody-antigen complex; and

35 (b) detecting the presence or absence of the antibody-antigen complex.

112. A method for detecting the presence of HCV particles in a sample suspected of containing HCV, comprising:

5 (a) incubating the sample with the binding complex of claim 109, wherein the incubating is conducted under conditions which allow for formation of an antibody-antigen complex; and

(b) detecting the presence or absence of the antibody-antigen complex.

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113. A method for detecting the presence of HCV particles in a sample suspected of containing HCV, comprising:

15 (a) incubating the sample with the binding complex of claim 110, wherein the incubating is conducted under conditions which allow for formation of an sFv-antigen complex; and

(b) detecting the presence or absence of the sFv-antigen complex.

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114. A method for monitoring the progress of an anti-HCV therapeutic treatment of a HCV-infected mammalian subject, comprising:

25 (a) obtaining a biological sample from said subject;

(b) incubating the sample with the binding complex of claim 108, wherein the incubating is conducted under conditions which allow for formation of an antibody-antigen complex; and

30 (c) detecting the presence or absence of the antibody-antigen complex.

115. A method for monitoring the progress of an anti-HCV therapeutic treatment of a HCV-infected mammalian subject, comprising:

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(a) obtaining a biological sample from said subject;

(b) incubating the sample with the binding complex of claim 109, wherein the incubating is conducted under conditions which allow for formation of an antibody-antigen complex; and

(c) detecting the presence or absence of the antibody-antigen complex.

116. A method for monitoring the progress of an anti-HCV therapeutic treatment of a HCV-infected mammalian subject, comprising:

(a) obtaining a biological sample from said subject;

(b) incubating the sample with the binding complex of claim 110, wherein the incubating is conducted under conditions which allow for formation of an sFv-antigen complex; and

(c) detecting the presence or absence of the sFv-antigen complex.

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